Communications of the Committee for Requirement Standards of the Society of Nutrition Physiology
Mitteilungen des Ausschusses für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie

Equations for predicting metabolisable energy and digestibility of organic matter in forage legumes for ruminants

Gleichungen zur Schätzung der Umsetzbaren Energie und der Verdaulichkeit der Organischen Substanz von Grobfutterleguminosen für Wiederkäuer

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1. Introduction

In recent years, the cultivation of forage legumes such as lucerne, red and white clover has become more important to obtain roughage sources. This is particularly relevant not only for supplying ruminants with farm-produced protein, but also for improving the supply of feed to induce an adequate structural fibre effect. When forage legumes are included in ration planning, its energy value must be adequately predicted. Evaluation of the data described below had suggested that the metabolisable energy (ME) of forage legumes cannot be predicted with sufficient precision based on the equations recommended for predicting the ME of grass and grass products (GfE 2008), and prediction equations were therefore derived specifically for forage legumes. This work was based on data sourced from digestibility experiments conducted in various institutions and regions in Germany and Switzerland with lucerne, sanfoin, red and white clover with wide variations in chemical composition (Losand et al. 2013). Equations for predicting the digestibility of organic matter (DOM) were additionally developed.

2. Data and procedures

A total of 89 data sets obtained from digestibility experiments conducted in seven institutions were used in the evaluation (Table 1). The digestibility of nutrients in freshly harvested forage, silage, hay and dried forage was determined in sheep according to the guidelines of the Society of Nutrition Physiology (GfE 1991) over a period extending from 2000 to 2014. The ME was calculated from the digestible crude nutrients using the following equation (GfE 1995, 2001):

$$
\begin{aligned}
\text{ME (MJ/kg)} = {} & 0.0312 \cdot \text{digestible crude fat (g/kg)} \\
& + 0.0136 \cdot \text{digestible crude fibre (g/kg)} \\
& + 0.0147 \cdot \text{digestible organic residue (digestible organic matter} \\
& \quad \text{- digestible crude fat - digestible crude fibre) (g/kg)} \\
& + 0.00234 \cdot \text{crude protein (g/kg)}
\end{aligned}
$$

Abbreviations used: **ADFom** = acid detergent fibre expressed exclusive of residual ash; **ADL** = acid detergent lignin; **aNDFom** = amylase-treated neutral detergent fibre expressed exclusive of residual ash; **CA** = crude ash; **CF** = crude fibre; **CL** = crude fat; **CP** = crude protein; **DM** = dry matter; **DOM** = digestibility of organic matter; **ESOM** = enzyme soluble organic matter; **GE** = gross energy; **GP** = gas production; **ME** = metabolisable energy; **NEL** = net energy for lactation; **NfE** = nitrogen-free extracts; **OM** = organic matter; **R^2** = coefficient of determination; **s%** = coefficient of variation; **v_R** = residual variance

Table 1: Number of data sets by origin, type of preservation and cut number

Origin/ Institution	Total	Type of preservation			Cut number		
		Fresh	Silage	Hay/ dried forage	1st cut	Regrowth	Not stated
ALP Posieux	20	7	4	9	7	11	2
Bingen	8		8		4	4	
Dummerstorf	35	15	12	8	9	24	2
Grub	16		16		6	6	4
Halle	1		1				1
Köllitsch	4	2		2			4
Kleve	5			5			5

The full data material from 89 data sets was used in the mathematical derivation of the estimating equations. For validation purposes, the derived equations were either applied separately to the categories forage, silage and hay/dried forage, and 1st cut, regrowth and 'Not stated' respectively, or to the full data material. Table 2 summarises the data from the comprehensive characterisation of the material.

Table 2: Composition[1)], *in vitro* criteria, digestibility of organic matter and calculated ME content of total material (mean, standard deviations, coefficient of variation s%, minimum and maximum).

		n	Mean	s	s%	Min.	Max.
Crude ash		89	120	34.5	28.7	69	282
Crude protein		89	194	34.9	18.0	112	276
Crude fat		89	23	7.7	34.1	5	38
Crude fibre	g/kg DM	89	265	64.0	24.1	114	382
aNDFom		84	402	89.8	22.4	185	600
ADFom		84	317	62.9	19.8	141	412
Lignin (ADL)		21	63	10.8	17.2	36	83
Gas production	ml/200 mg DM	54	41.6	5.8	14.0	29.7	51.3
ESOM	g/kg DM	82	597	64.7	10.8	459	741
DOM	%	89	67.5	7.0	10.4	53.5	82.8
ME	MJ/kg DM	89	9.21	0.9	9.3	7.5	11.3

[1)] Silage values except gas production corrected for e losses of volatile substances during drying.

With coefficients of variation between 20 and 24% for the fibre fractions, of 18% for crude protein, of 34% for crude fat, and of approximately 10% for DOM and ME contents respectively, the data exhibited sufficient variance for deriving regression equations. The lignin content (determined as acid detergent lignin/ADL) was only stated in some of the data sets, and this fraction was therefore not taken into account in the regression calculations. However, the relevant values have been included in Table 2 for reasons of completeness and as a basis for possible future updates. The values for crude fibre contents are also stated in order to characterise the material in more detail, and they are required for calculating the reference values for ME. However, crude fibre was not taken into account as a variable in deriving the estimating equations. The estimating equations were derived from the ME contents, the contents of nutrients and enzyme soluble organic matter (ESOM), and the gas production (GP) of the organic matter. Validation was, however, based on the converted ME content relative to dry matter, and standard error and bias are also stated on this basis.

As a first step, various estimating equations for predicting ME contents were derived and subsequently compared based on their coefficient of determination (R^2), residual variance (v_R) and bias. Initially it was intended to derive a single estimating equation that would be valid for all of the forage legumes. However, this did not yield satisfactory results with all types of preservation and cut numbers, and separately derived estimating equations were therefore examined for the 1st cut and regrowth, similar to earlier approaches for grass and forage products (GfE 1998). As a third step, the derived estimating equations were validated using the full data pool, separated by type of preservation and cut number. The equations were derived using the ***Proc Reg*** procedure of the SAS® statistical software package with stepped parameter selection. The following variables were considered, using a constant additive value: crude protein, crude fat, ADFom or aNDFom respectively, ESOM and GP, relative to the content in organic matter. Conceptionally, variants were first calculated using one of the two *in vitro* parameters and then also used together in another derivation due to the relatively low correlation between ESOM and GP ($r = 0.64$). Only variables with a level of significance of $p < 0.15$ were taken into account.

3. Results

3.1 Regression equations for calculating ME

Table 3 summarises the results of the evaluation. The inclusion of aNDFom was not helpful in any case, as the level of significance of the regression coefficient was consistently substantially below that for ADFom. Equations 1-3 have been derived based on the full data pool without special consideration of the cut number. However, these equations are only comparable to a limited degree in terms of the accuracy of the estimate they allow (R^2, v_R), as they are each based on data pools of different sizes. With an already relatively high coefficient of determination of $R^2 = 0.84$ and a low residual variance v_R of 0.47 or 0.50 MJ/kg OM respectively, the reliability of the estimated ME content of forage legumes is improved even further by using both ESOM and GP together. This also applies similarly to the separate ME content estimates for the 1st cut and regrowth and the category without stated cut number respectively. The materials for which the cut number was not stated were counted among the regrowth cuts in the evaluation and not among the 1st cut.

Table 3: Coefficient of determination (R^2) and residual variance (v_R) of the derived estimating equations for the ME content of organic matter

Equation No.	n	Variables	Cut number	R^2	v_R
ME_1	81	ADFom, CP, CL, ESOM	Not differentiated	0.84	0.47
ME_2	54	ADFom, CP, CL, GP	Not differentiated	0.84	0.50
ME_3	53	ADFom, CP, CL, ESOM, GP	Not differentiated	0.89	0.43
ME_1_1	22	ADFom, CP, CL, ESOM	1	0.90	0.36
ME_2_1	17	ADFom, CP, CL, GP	1	0.89	0.42
ME_3_1	16	ADFom, CP, CL, ESOM, GP	1	0.89	0.36
ME_1_2	58	ADFom, CP, CL, ESOM	Regrowth and not stated	0.85	0.46
ME_2_2	36	ADFom, CP, CL, GP	Regrowth and not stated	0.86	0.50
ME_3_2	36	ADFom, CP, CL, ESOM, GP	Regrowth and not stated	0.91	0.41

3.1.1 Validation of the prediction equations for ME

Validation was performed for the full data pool, separately for the categories forage, silage and hay, and separately for 1st cut, regrowth and data material without indication of cut number (Table 4), using the leave-one-out cross-validation approach. Independent validation was not possible due to the limited number of samples in the various categories following differentiation. It was noted that the selection of the *in vitro* parameter is irrelevant for the accuracy of the estimate, as the standard error remains approximately the same. Where analytical results are available for both *in vitro* parameters, the accuracy of estimate can be improved further. In terms of the type of preservation, the robustness achieved with a general equation that does not take this aspect into account was only given for ESOM. Inclusion of the GP parameter for the data pool (which was not, however, identical) revealed that the application of the estimating equation results in substantial underestimation for fresh forage. However, the considerable, general underestimate of the 1st growth ME content by more than 0.2 MJ ME/kg DM that resulted from the use of equations 1-3 was more remarkable. Thus, there were differences between crops from the 1st cut and from regrowths throughout the year, which are not represented by commonly used analytical variables, even when the two *in vitro* digestibility characteristics are included. Where estimating equations are derived separately for 1st cut and regrowth materials, this biased deviation is largely neutralised. However, even in this case a systematic underestimate of the ME content of forage persists when GP is used.

Table 4: Standard error (%) and bias in the validation of the derived estimating equations for calculating ME content (MJ ME/kg DM), differentiated by type of preservation and cut number

Equation	Parameter	All	Type of preservation			Cut number		
			Fresh	Silage	Dried	1	Regrowth	Not stated
1	n	81	24	34	23	23	42	16
	Mean	9.22	9.78	9.12	8.78	9.55	9.22	8.73
	% standard error	4.4	5.2	4.3	3.3	3.5	4.4	3.7
	Bias	0.00	-0.04	-0.04	0.11	-0.25	0.05	0.24
2	n	54	13	19	22	18	24	12
	Mean	9.11	9.90	8.85	8.88	9.41	9.06	8.78
	% standard error	4.7	2.5	5.3	4.6	3.7	4.4	5.4
	Bias	0.00	-0.28	0.03	0.14	-0.23	0.06	0.23
3	n	53	13	19	21	17	24	12
	Mean	9.11	9.97	8.86	8.80	9.45	9.07	8.71
	% standard error	4.0	3.4	4.6	3.4	3.6	4.0	3.2
	Bias	0.00	-0.20	0.04	0.09	-0.20	0.06	0.16
1_1/1_2	n	81	24	34	23	23	42	16
	Mean	9.21	9.77	9.16	8.76	9.80	9.13	8.63
	% standard error	4.3	5.0	4.0	3.9	3.6	4.6	4.3
	Bias	0.01	-0.03	-0.02	0.08	0.01	-0.05	0.14
2_1/2_2	n	54	13	19	22	18	24	12
	Mean	9.15	9.92	8.94	8.92	9.70	8.97	8.70
	% standard error	5.6	2.7	7.0	4.9	6.1	47.6	6.7
	Bias	0.04	-0.26	0.07	0.18	0.01	-0.03	0.16
3_1/3_2	n	53	12	20	21	17	24	12
	Mean	9.11	9.97	8.93	8.78	9.63	8.96	8.65
	% standard error	4.5	4.0	5.1	3.9	4.8	4.3	4.3
	Bias	0.00	-0.21	0.05	0.07	-0.01	-0.04	0.10

Figure 1 shows the differences between the ME content estimates based on equation 1 and the values calculated from digestible crude nutrients. Based on equation 1, the residual variance of the estimated ME content remains about constant across almost the entire range of variation. Figure 2, in contrast, clearly indicates the negative deviations of the ME content estimates of 1st cut material based on equation 1. In equation 1, the underestimated ME content is offset by the tendency towards over-estimating materials without stated cut number. Where 1st cut materials are evaluated separately, this estimate error for 1st cut materials and materials without stated cut number is no longer present (Figure 3).

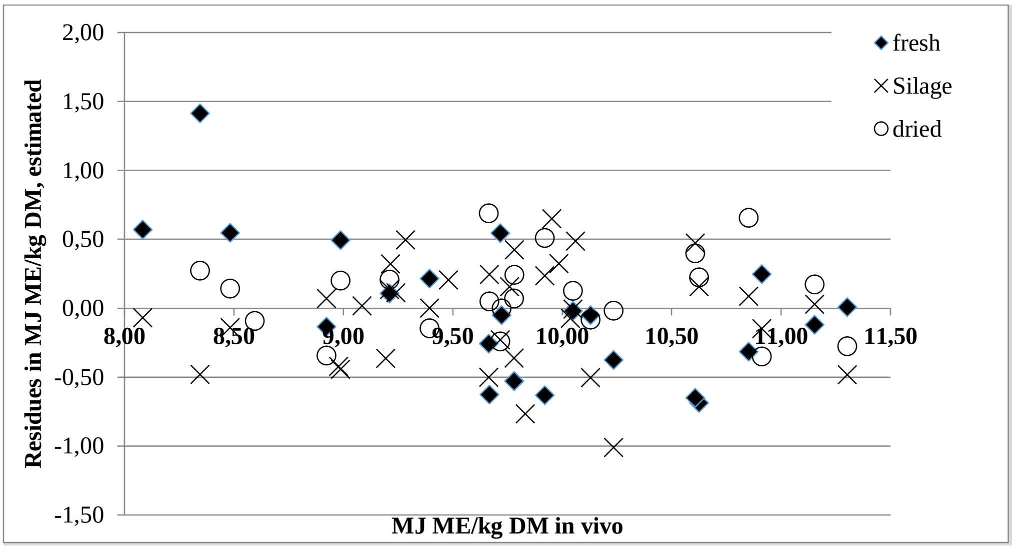

Figure 1: Differences between estimated ME contents and ME contents determined in digestibility trials with sheep by type of preservation (equation ME_1)

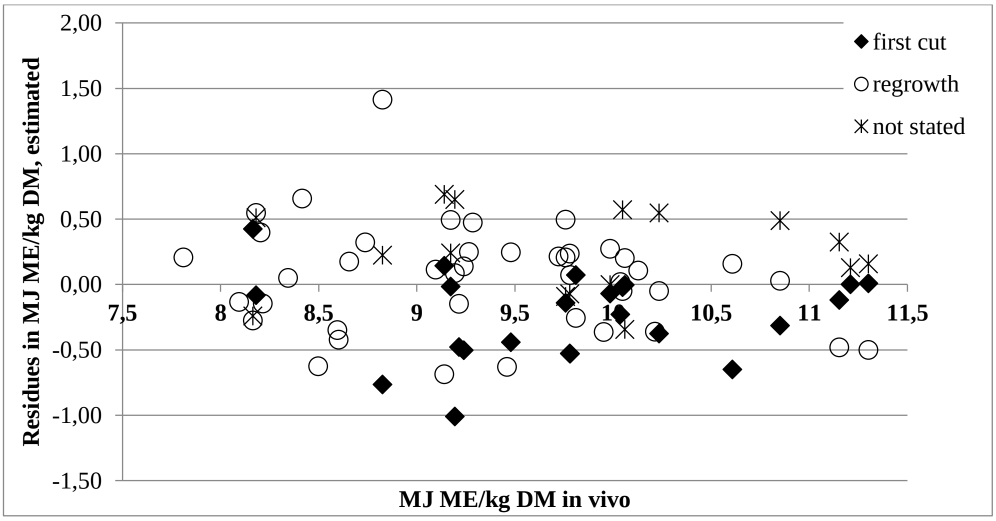

Figure 2: Differences between estimated ME contents and ME contents determined in digestibility trials with sheep by cut number (equation 1)

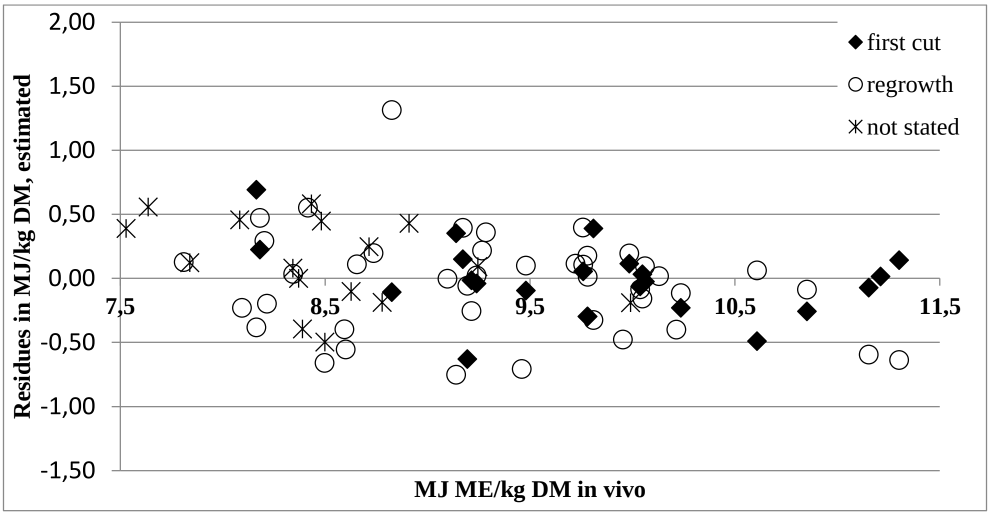

Figure 3: Differences between estimated ME contents and ME contents determined in digestibility trials with sheep by cut number (equations ME_1_1 and ME_1_2)

3.1.2 Recommended equations for predicting ME in forage legumes

In line with the procedure for grass and maize products (GfE 2008) and compound feeds for ruminants (GfE 2009), the Committee recommends the use of two alternative equations for predicting ME in forage legumes, taking into account either ESOM or GP (Table 5). However, with both alternatives a separate equation needs to be used for the 1st cut in order to improve the accuracy of the estimate. A different equation is consequently recommended for regrowth. If no information is provided on the cut number, the equation for regrowths should be used; this results in 1st cut samples not marked as such being underestimated by about 0.3 MJ ME/kg DM. In order to avoid this systematic error it is therefore important that samples sent in for evaluation are clearly and routinely marked with the cut number.

Table 5: Recommended equations for predicting the ME content of organic matter in forage legumes

Based on ESOM

First cut			Regrowth and without stated cut number		
ME =	11.91		ME =	9.83	
-	0.01034	• ADFom	-	0.01010	• ADFom
+	0.00389	• CP	+	0.00039	• CP
+	0.01870	• CL	+	0.00802	• CL
+	0.00191	• ESOM	+	0.00571	• ESOM
R^2 =	0.904		R^2 =	0.852	
v_R =	0.36		v_R =	0.46	

ME in MJ/kg OM; CP, CL, ADFom, ESOM in g/kg OM

Based on gas production

First cut			Regrowth and without stated cut number		
ME =	12.49		ME =	11.09	
-	0.01140	• ADFom	-	0.01040	• ADFom
+	0.00425	• CP	+	0.00497	• CP
+	0.02690	• CL	+	0.00750	• CL
+	0.01683	• GP	+	0.0351	• GP
R^2 =	0.891		R^2 =	0.862	
v_R =	0.42		v_R =	0.50	

ME in MJ/kg OM; CP, CL, ADFom in g/kg OM; GP in ml/200 mg OM

The following equation was then used to convert values into ME content in dry matter for specific samples for evaluation based on their organic matter content:

ME (MJ/kg DM) = ME (MJ/kg OM) • [1000 – CA (g/kg DM)]/1000

For dairy cows, net energy lactation (NEL) can be calculated from ME as follows, taking into account gross energy (GE) metabolisability (q) according to GfE (2001):

NEL (MJ) = 0,6 [1 + 0.004 (q – 57)] ME (MJ), with q = ME/GE • 100

If the GE content has not already been measured by bomb calorimetry, it must initially be determined using the following equation:

GE (MJ/kg DM) = 0.0239 • CP (g/kg DM) + 0.0398 • CL (g/kg DM) + 0.0201 • CF (g/kg DM
+ 0.0175 • NfE (g/kg DM)

For this calculation, the content of crude fibre and N-free extracts (NfE) must be known. If this is not the case, ME can be converted into NEL contents from the following, simplified equation (Weißbach et al. 1996):

NEL (MJ/kg DM) = ME [0.46 + 12.38 • ME / (1000 – CA)], with: ME in MJ/kg DM; CA in g/kg DM

3.2 Regression equations for predicting DOM

Crude protein, crude fat, ADFom, aNDFom and at least one *in vitro* criterion were also used to derive the estimating equations for DOM. The calculations were made using concentrations in organic matter. Parameters were excluded as being insignificant if their regression coefficients did not reach a significance level of $p < 0.15$. Similar to the approach taken in predicting ME, a single equation was initially derived for all types of preservation and cut numbers. A separate equation was then derived in a second step for 1st cut materials. Table 6 summarises the results of the regression analyses.

Table 6: Coefficient of determination (R^2) and residual variance (v_R) of the derived estimating equations for calculating digestibility of organic matter (in %)

Equation No.	n	Variables	Cut number	R^2	v_R
DOM_1	81	ADFom, CP, ESOM	Not differentiated	0.82	3.17
DOM_2	54	ADFom, GP	Not differentiated	0.82	3.37
DOM_3	53	ADFom, ESOM, GP	Not differentiated	0.87	2.93
DOM_1_1	22	ADFom, ESOM	1	0.89	2.31
DOM_2_1	17	ADFom, GP	1	0.88	2.76
DOM_3_1	16	ADFom, ESOM, GP	1	0.89	2.72
DOM_1_2	58	ADFom ESOM	Regrowth and not stated	0.81	3.23
DOM_2_2	36	ADFom, GP	Regrowth and not stated	0.83	3.36
DOM_3_2	36	ADFom, ESOM, GP	Regrowth and not stated	0.88	2.91

This table shows a clear reduction in the number of independent variables compared to the ME contents estimate (Table 3), which is associated with a higher weighting of the *in vitro* parameters for predicting DOM compared to predicting ME. By using both *in vitro* criteria, the accuracy of the estimate could be somewhat improved compared to the alternative use of a single criterion only.

3.2.1 Validation of the prediction equations for DOM

Validation was again performed based on the entire data pool and the sub-categories (Table 7) using the leave-one-out cross-validation approach. As with the approach taken in predicting ME, the use of only one equation in estimating DOM across all cut numbers and types of preservation consistently results in biased negative deviations of 1.5 to 1.8 percentage points for 1st cut materials and positive deviations for materials without stated cut numbers, above all. There are additionally inaccuracies in differentiating DOM in fresh and dried crops when using the GP equation (equation DOM_2). Where estimating equations are derived separately for 1st cut and regrowth materials, there is no such biased deviation (equations DOM_1_1+2 to DOM_3_1+2; Figure 4). However, even in this case a biased underestimate persists for forage when GP is used.

Table 7: Standard error and bias in applying the derived equations for predicting digestibility of organic matter (DOM, %) based on the complete data pool and on sub-categories

Equation	Parameter	All	Type of preservation			Cut number		
			Fresh	Silage	Dried	1	Regrowth	Not stated
DOM_1	n	81	24	34	23	23	42	16
	Mean	67.7	72.0	66.1	65.6	69.3	68.6	63.2
	% standard error	4.6	5.6	4.2	3.7	3.4	4.7	4.5
	Bias	-0.01	-0.53	0.11	0.37	-1.68	0.35	1.44
DOM_2	n	54	13	19	22	18	24	12
	Mean	67.3	74.4	63.4	67.1	68.4	68.4	63.6
	% standard error	4.9	3.0	5.3	4.8	4.0	4.5	5.8
	Bias	0.00	-2.29	0.07	1.18	-1.69	0.48	1.58
DOM_3	n	53	13	19	21	17	24	12
	Mean	67.3	74.6	63.7	66.0	68.6	68.4	63.2
	% standard error	4.2	3.7	4.9	3.5	3.5	4.0	4.7
	Bias	0.01	-1.73	0.80	0.36	-1.47	0.46	1.17
DOM_1_1/ DOM_1_2	n	81	23	35	23	23	42	16
	Mean	67.7	72.1	66.3	65.4	71.0	67.8	62.6
	% standard error	4.7	5.5	4.4	4.2	3.6	4.9	5.5
	Bias	-0.02	-0.50	0.20	0.1	0.04	-0.39	0.83
DOM_2_1/ DOM_2_2	n	54	12	20	22	18	24	12
	Mean	67.4	74.9	63.5	66.7	70.0	67.6	62.9
	% standard error	5.0	3.6	5.1	5.4	4.4	4.9	6.2
	Bias	-0.02	-1.77	0.15	0.88	-0.06	-0.34	0.85
DOM_3_1/ DOM_3_2	n	53	12	20	21	17	24	12
	Mean	67.1	75.0	63.7	65.8	69.6	67.6	62.4
	% standard error	4.4	4.1	4.3	4.4	3.7	4.4	5.4
	Bias	-0.20	-1.72	0.31	0.18	-0.46	-0.32	0.4

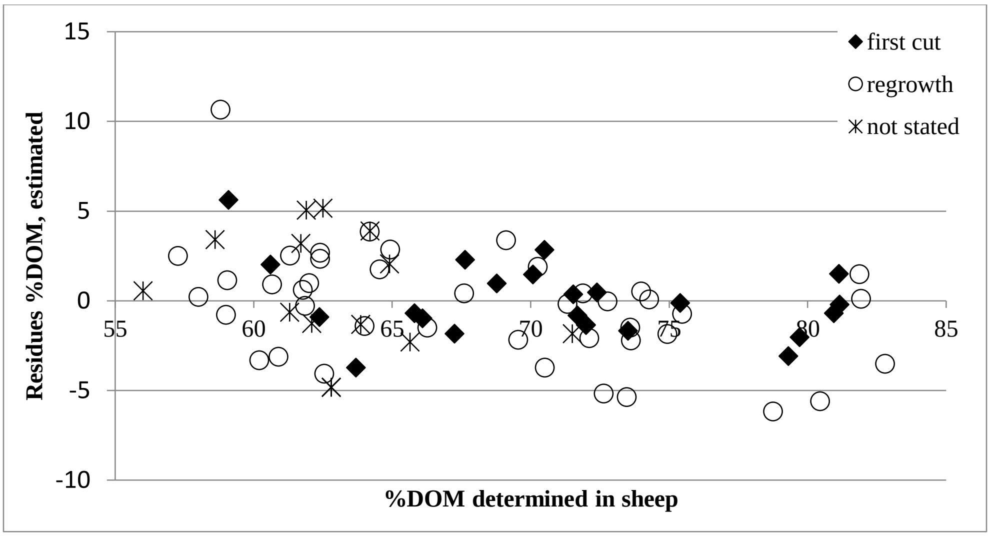

Figure 4: Residues of the estimated digestibility of organic matter (DOM) based on ESOM, compared to values determined in digestibility trials with sheep, by cut number (equations DOM_1_1 and DOM_1_2)

3.2.2 Recommended equations for predicting DOM in forage legumes

The Committee also recommends the use of two alternative equations for predicting DOM in forage legumes, using ESOM and GP and differentiating by cut number (Table 8). According to both recommendations, a separate equation should be used for the 1st cut, and the relevant regrowth equation should be used for regrowth. The equation for regrowth should be used where no information is available on the cut number. Applying the second equation to 1st cuts that are not marked as such results in an underestimate of digestibility by about 2.5 percentage points.

Table 8: Recommended equations for predicting DOM in forage legumes

Based on ESOM					
First cut			Regrowth and without stated cut number		
DOM =	81.71		DOM =	70.77	
-	0.0711	• ADFom	-	0.0683	• ADFom
+	0.0195	• ESOM	+	0.0302	• ESOM
R^2 =	0.892		R^2 =	0.806	
v_R =	2.31		v_R =	3.23	
DOM in %; ADFom ESOM in g/kg OM;					
Based on gas production					
First cut			Regrowth and without stated cut number		
DOM =	95.72		DOM =	77.90	
-	0.0859	• ADFom	-	0.0711	• ADFom
+	0.0964	• GP	+	0.2997	• GP
R^2 =	0.859		R^2 =	0.832	
v_R =	2.813		v_R =	3.36	
DOM in %; ADFom in g/kg OM; GP in ml/200 mg OM					

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